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GENOME ANNOUNCEMENT

Complete Genome Sequence of the English Isolate of Rat Cytomegalovirus (*Murid Herpesvirus 8*)

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The complete genome of the English isolate of rat cytomegalovirus (RCMV-E) was determined. RCMV-E has a 202,946-bp genome with noninverting repeats but without terminal repeats. Thus, it differs significantly in size and genomic arrangement from closely related rodent cytomegaloviruses (CMVs). To account for the differences between the rat CMV isolates of Maastricht and England, RCMV-E was classified as *Murid herpesvirus 8* by the International Committee on Taxonomy of Viruses.

The English isolate of rat cytomegalovirus (RCMV-E) is a member of the *Betaherpesvirinae* subfamily of the *Herpesviridae*. Two isolates of RCMV, the Maastricht isolate (RCMV-M) and RCMV-E, have been reported, and RCMV-M has been classified as *Murid herpesvirus 2* (MuHV-2). RCMV-M was first described in 1982 by Bruggeman et al. (2), and in the same year, Priscott and Tyrrell (4) reported on the existence of another rat CMV that was later termed the "English" isolate (3). Both viruses had been isolated from *Rattus norvegicus*.

The complete genome sequences of both MuHV-2 and MuHV-1 (MCMV Smith strain) have been published (NC_002512, 230,138 bp [7] and NC_004065, 230,278 bp [5]) as well as those of four very closely related isolates of MuHV-1 (6). The RCMV-M and RCMV-E genomes were shown to have significantly different restriction enzyme cleavage patterns, suggesting that they represent different betaherpesvirus species rather than different strains of the same virus (1, 8).

To analyze the viral genome, RCMV-E virion DNA was isolated from RCMV-E-infected rat embryo fibroblasts and analyzed by Sanger sequencing. In addition, shotgun sequencing (Macrogen, South Korea) with low coverage was performed. These data revealed substantial differences to MuHV-2 and served as a scaffold sequence. To confirm the data, RCMV-E virion DNA was subjected to 454 sequencing. The DNA was sheared with Covaris S2, and libraries were generated utilizing the rapid library kit and finally sequenced with Titanium chemistry on a 454 FLX instrument (Roche). Reads with a 26-fold coverage were mapped to our scaffold sequence data using Newbler 2.6 software. Analysis of the data showed that the RCMV-E genome comprises 202,946 bp. Results of a *de novo* assembly using Newbler 2.6 confirmed the results of the mapping. Therefore, from both physical analysis and the genome sequencing data, it is evident that the RCMV-E genome is considerably smaller than and thus differs significantly in size and gene content from both MuHV-1 and MuHV-2.

Major criteria to identify open reading frames (ORFs) were a minimum length of 60 bp, an ATG start codon, and less than 60% overlap with adjacent ORFs. By this approach, 140 ORFs were identified using Lasergene 8 and Geneious 5.4 software packages. A total of 118 ORFs are homologous to MuHV-1 and MuHV-2, showing an E value of <0.001 in BLASTX analyses. Both at the nucleotide and protein levels, most RCMV genes are somewhat more closely related to MuHV-1, although they are almost equally divergent from both MuHV-1 and MuHV-2. However, RCMV-E encodes 22 additional ORFs, 19 of which have no obvious homologue in either virus. Within individual coding regions, amino acid sequence identity ranges from 10 to 87% between MuHV-1 and RCMV-E and from 10 to 84% between MuHV-2 and RCMV-E. These data confirm that RCMV-E, now classified as *Murid herpesvirus 8*, is a separate species within the *Betaherpesvirinae* (http://ictvonline.org/virusTaxonomy.asp?version=2011).

Nucleotide sequence accession number. The RCMV-E genome sequence has been deposited in GenBank under accession number JX867617.

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